REMARKS

As an initial matter, Applicants thank the Examiner for the courtesy extended during the telephonic discussion on December 3, 2003. Applicants appreciate the opportunity to discuss the issues in the application.

Upon entry of the amendment, claims 1-2, 4-5, 7-13, 15, 37, 39, 42-45 and 47-57 will be pending in the application. Claims 6, 38 and 40-41 are cancelled with the present amendment and new claims 48-57 are added. Claims 1, 4, 5, 7-12, 37, 39 and 42-44 are amended. Support for the amendments to claims 1 and 37 appears in now cancelled claims 6 and 38 (reciting that the reduction in the capacity of the cells to utilize copper is effected by a transition metal chelator having an affinity for copper) and in now cancelled claims 40-41 (disclosing that the conditions for cell proliferation can include providing nutrients early acting cytokines). The remaining amendments more clearly point out the subject matter claimed, address various informalities, or clarify antecedent bases (e.g., the amendments to claims 1, 11, 37, and 43 specifying 'cytokine or cytokines' clarify the bases for dependent claims 10, 12, 42, and 44). Support for new claims 48 and 49 appears in, e.g., previous claims 1 and 37. Support for new claims 50 and 51 appears in the specification at page 8, lines 24-25 and 34-36; page 12, lines 4-11 and page 19, lines 14-21 and support for new claims 52-57 appears in the specification at page 25, lines 31-34. No new matter is added by these amendments. The amendment or cancellation of claims does not constitute an admission by Applicants that the subject matter no longer claimed is not patentable. Applicants reserve the right to pursue all cancelled subject matter in a continuing application or applications.

The claimed invention provides methods for expanding a population of undifferentiated hematopoietic cells for transplantation, while at the same time inhibiting their differentiation, by adding a transition metal chelator having an affinity for copper. Among the advantages of the method is more efficient expansion of a population of hematopoietic cells.

Rejection under 35 U.S.C. 112, First Paragraph

Claims 1-2, 4-13, 15, 37-45 and 47 are rejected under 35 U.S.C. 112, first paragraph, for lack of enablement. Claims 6, 38 and 40-41 are cancelled. The rejection is traversed to the extent it is applied to the remaining claims as amended.

Independent claim 1, from which depends claims 2, 4-13, and 15, is drawn to a method of transplanting hematopoietic cells. Similarly, claim 37, from which depends claims 38-45 and 47, is drawn to a method of adoptive immunotherapy using hematopoietic cells.

The Examiner acknowledges that the specification enables claims drawn to $CD34^+$ cells that are first isolated (*i.e.* enriched) and then used for *ex vivo* expanding, but contends that the specification is not enabled for any hematopoietic cells (*See*, Office Action at page 3).

Applicants respectfully disagree.

Applicants respectfully submit that the full breadth of the invention now claimed can be practiced using the knowledge available to one of ordinary skill in the art when coupled with the teachings of the specification. For example, the specification teaches that transition metal chelators are effective in enhancing expansion and inhibiting differentiation of hematopoietic cell types in addition to those that are CD34+ selected. Pages 37, line 10 to page 38, line 10, and Table 2 of Example 1 of the specification teaches that transition metal chelators stimulate expansion while inhibiting differentiation of murine erythroleukemia cell cultures as well as undifferentiated CD34+ cells. Other examples of growth induction and inhibition of cell differentiation by transition metal chelators in diverse cell populations at various stages of differentiation are also taught in the specification (*See*, for example, Table 2- Effect of TEPA on murine erythroleukemia cells; Figure 5, effect of TEPA on erythroid precursors form peripheral blood mononucleocytes; Example 4, Table 4, Effect of TEPA on embryonal stem cells; and page 48, Example 4- Effect of TEPA on hepatocyte growth and differentiation).

The specification additionally teaches that hematopoietic reconstitution of lethally irradiated mice with bone marrow cells expanded *ex vivo* with the transition metal chelator TEPA and cytokines results in superior WBC recovery and survival as compared to cells expanded with cytokines alone (*See*, Example 5, pages 51 and 52, and Table 7).

Thus, contrary to the Examiner's contention, the specification discloses methods for *ex vivo* expansion of and inhibition of differentiation of stem/progenitor cells from a broad range of cells in addition to CD34+ cells. The instant specification is clearly enabling for expanded hematopoietic cells in addition to those that are CD34+, or cells selected on the basis that they are CD34+. The specification fulfills the requirement for "a recitation of a representative number of cell types falling within the scope of the (claimed) genus", as indicated by the Examiner (*See* Office Action at page 5, paragraph 1).

The teachings of the specification have also been used to practice the claimed invention for hematopoietic cells that are not CD34+ selected. One example is illustrated in the attached Declaration of Eitan Fibach ("the Fibach Declaration"), which discusses the use of the claimed methods on AC133 cells. AC133 cells have high self-renewal capability, maintain early hematopoietic stem/progenitor cell (HSPC) characteristics, and show superior survival in culture, as compared to CD34+ cells.

The Fibach Declaration demonstrates that following a three week large-scale clinical grade expansion, the yield of cord blood-derived early progenitor cells (TNC, CFUc, CD34+ cells and CD34+CD38- cells) in high affinity copper chelator-supplemented (5 μM TEPA) culture initiated with AC133+ cells was statistically similar with that initiated with a same number of CD34+ cells. In addition, similar proportions of cells expressing myeloid, lymphoid and megakaryocytic phenotype were found in cultures initiated either with CD34+ or AC133+ cells.

Thus, in addition to CD34+ cells, the claimed methods can be used on the additional cell types disclosed in the specification and AC 133+ cells cultured in the presence of a transition metal chelator efficient expansion and inhibition of differentiation of hematopoietic stem/progenitor cells. These results clearly indicate that: (i) the methods of the present invention can be successfully extrapolated to populations of undifferentiated cells other than CD34+ cells; (ii) using the methods taught in the instant specification, one of ordinary skill in the art would expect, with a reasonable degree of success, to effectively expand and inhibit differentiation of a broad range of undifferentiated hematopoietic cells.

The Examiner has further stated that the present invention "must be considered unpredictable" since the mechanism of the effects of copper are as yet unknown. To the contrary, elucidation and knowledge of the mechanism of a drug's biological activity is hardly an acceptable criterion for predictability of the activity of the drug. Examples abound in which drugs are approved and routinely used for specific therapeutic effects, the pathways and mechanisms of which are poorly understood (aspirin and pain relief is only one example). Although understanding of the effects of copper on the process of differentiation will undoubtedly be of great value, demonstration of the predictable effects of transition metal chelators on expansion and inhibition of differentiation of hematopoietic cells, as taught in the instant specification, need not depend on such understanding. Further, the Examiner has stated that "the scope of the claims must bear a reasonable correlation with the scope of enablement". The examples brought hereinabove provide evidence for clear enablement for expansion and inhibition of differentiation of diverse types of undifferentiated cells to be found in the instant specification.

In view of the foregoing comments, Applicants submit the pending claims as amended herein are fully enabled by the specification, and respectfully request this rejection be withdrawn.

Rejections under 35 U.S.C. 103(a)

Claims 1-2, 4-5, 8-13, 15, 37, 40-45 and 47 are rejected as obvious over Moore et al, Blood Cells, 20: 468-48, 1994 ("Moore"); or De Bruyn et al., Stem Cells 13: 281-288, 1995 ("De Bruyn"), each in view of Cicuttine et al. Blood 80: 102-112 (1992) ("Cicuttine"). Claims 40 and 41 have been cancelled. The rejection is traversed to the extent it is applied to the remaining claims as amended.

Claim 1, from which depends claims 2, 4-5, 8-13, and 15, has been amended to incorporate the subject matter of claim 6, which is not subject to the rejection. Similarly, independent claim 37, from which depends claims 42-45 and 47, has been amended to incorporate the subject matter of claim 38, which also is not subject to the rejection. Therefore, all of the claims as amended now are drawn to subject matter that was not subject to the

rejection. Therefore, the rejection of claims 1-2, 4-5, 8-13, 15, 37, 40-45 and 47 as obvious over Moore, De Bruyn, and Cicuttine can be withdrawn.

Claims 6-7 and 38-39 are rejected as obvious Moore or De Bruyn each in view of Percival , J. Nutrition 122: 2424-2429 (1992) ("Percival I"). The rejection is traversed to the extent it is applied to the claims as amended.

Claims 6 and 38 have been cancelled and their subject matter incorporated into claims 1 and 37, respectively (claims 7 and 39 are now amended to depend from claim 1 and 37, respectively). Applicants will address the present rejection to the extent that it applies to claims 1 and 37 amended herein.

Independent claims 1 and 37, from which the remaining clams subject to the rejection depend, require expanding hematopoietic cells *ex vivo* in the presence of a transition metal chelator, wherein the chelator inhibits differentiation of the cells.

As noted by the Examiner, neither Moore nor De Bruyn explicitly teach a method of hematopoietic cell transplantation or a method of adaptive immunotherapy using a transition metal chelator such as tetraethylenepentamine ("TEPA") (*See*, Office Action at page 7, sixth paragraph). Percival I is used to complete the rejection for stating, according to the Examiner, that copper is essential for the process of differentiation and that chelating copper with TEPA inhibits differentiation.

Applicants disagree with this characterization of Percival I. Percival I does not say that TEPA inhibits differentiation; rather, this reference reports instead that TEPA has no effect on differentiation. Percival I examined the ability of TEPA to make the leukemic cell line HL-60 copper deficient. The effects of adding TEPA to the HL-60 cell line were assessed by measuring cellular copper levels and the activity of the copper-containing enzymes Cu/An superoxide. The decrease in Cu/Zn-SOD activity was not attributed to a decrease in Cu/Zn-SOD protein levels, which would be an indicator of differentiation, but was instead attributed to chelation of the copper required for the enzyme to function. In addition, Percival I found that no increase in respiratory activity was detected following addition of the copper chelator. Percival I concluded from this TEPA did not affect differentiation:

> In summary, incubating HL-60 cells with TEPA resulted in copper-deficient cells without loss of viability and without alteration in the stage of differentiation.(page 2428, right column, last paragraph)

and:

> Respiratory burst activity, an indicator of differentiation, was not affected by TEPA, demonstrating that the reduction of Cu/Zn SOD activity was due to copper chelation and not due to changes in Cu/Zn SOD protein levels that occur during differentiation."(Abstract, page 2424, left column).

Moreover, one of ordinary skill in the art would also be aware of a later publication by Percival (Am. J. Clin. Nutr. 67:1064-68, 1998)("Percival II"), which was listed by the Examiner in the Notice of References Cited accompanying the February 11, 2003 Office Action. Percival II expressly states that copper chelation does not lead to differentiation of HL-60 cells (page 1066S):

> If copper is removed from the cell, is differentiation impaired or prevented? We hypothesized that if copper is essential for differentiation, then chelation of copper with TEPA should prevent the cells from differentiating. ...Cells incubated with TEPA and retinoic acid produced the same amount of superoxide anion as did the cells with retinoic acid, indicating that differentiation had occurred. . . . So whereas our TEPA model is useful in some studies related to manipulating copper concentrations and Cu/Zn SOD activity, it does not prevent the HL-60 cells from differentiating.

The artisan, reading Percival I and Percival II, would conclude that TEPA has no effect on differentiation. As noted above, the claims now expressly require that a transition metal chelator inhibit differentiation. The artisan would therefore have no motivation to use a transition metal chelator as described in either Percival I or Percival II in the method of Moore or De Bruyn to produce the claimed invention, which now requires expanding hematopoietic cells *ex vivo* in the presence of a transition metal chelator, wherein the chelator inhibits differentiation of the cells. Nor would there be any expectation after considering Percival I that the invention now claimed would be successful.

Applicants additionally note that the use of the HL-60 cell line in Percival I would cause the artisan to avoid combining this reference with Moore or De Bruyn, which describe methods of expanding cells for subsequent reintroduction into a host. The HL-60 cell line derived from a patient with promyelocytic leukemia, is known to propagate only *in vitro*, and as such cannot be

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SERIAL NUMBER: **09/463,320**

The Applicant in the above-identified application is a small entity, and is therefore entitled to claim status as a small entity. Please charge any additional fees that may be due, or credit any overpayment of same, to Deposit Account No. 50-0311, Reference No. 24024-501 CON .

Respectfully submitted,

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